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PCT

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference DK61998PC	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/04039	International filing date *(day/month/year)* 17.04.2003	Priority date *(day/month/year)* 17.04.2002
International Patent Classification (IPC) or both national classification and IPC A61K47/48		
Applicant DEUTSCHES KREBSFORSCHUNGSZENTRUM et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.11.2003	25.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Dullaart, A Telephone No. +31 70 340-3290

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/04039

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-21 as originally filed

Claims, Numbers

1-22 filed with telefax on 05.08.2004

Drawings, Sheets

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-22 in part

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 1-22 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☒ the claims, or said claims Nos. 1-22 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-22 in part

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☒ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-22 .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations

see separate sheet

Due to circumstances beyond our control, the present time limit for establishing the International Preliminary Examination Report has almost expired. In order to safeguard the rights of the applicant as much as possible, a rather short time-limit for replying to this communication of 1 month and 7 days has been set.
The International Preliminary Examination Authority apologises for any inconvenience caused.

Reference is made to the following documents:

D1: **WO 02 016418 A (Thomas Jefferson University, US) 28 February 2002**

D2: **WO 02 016402 A (Board of Regents, the University of Texas System, US) 28 February 2002**

D3: **WO 02 026775 A (Trustees of Princeton University, US) 4 April 2002**

D4: **WO 01 49719 A (Univ Texas System) 12 July 2001**

D5: **Database CA [Online] Chemical Abstracts Service, Columbus, Ohio, US; Endo, Hitoshi et al: 'Cysteine, basic and neutral amino acid transporter BAT1 from rat and human activated by rBAT, cDNA, and recombinant expression'**
retrieved from STN Database accession no. 134:174559 HCA XP 002204777 & JP 2001 046070 A (Foundation for Scientific Technology Promotion, Japan) 20 February 2001 (2001-02-20)

D6: **WO 00 58488 A (Dalby Brian; Invitrogen Corp (US); Bennett Robert P (US)) 5 October 2000**

D7: **WO 01 38547 A (Rosenecker Joseph ;Plank Christian (DE); Ritter Wolfgang (DE); Rud) 31 May 2001**

D8: **WO 00 29427 A (Cyclacel Ltd ;Fischer M Peter (GB); Zhelev Nikolai (GB)) 25 May 2000**

D9: **WO 99 05302 A (PERKIN ELMER CORP) 4 February 1999**

D10: **Derossi D et al: 'Trojan Peptides: the Penetratin System for Intracellular Delivery'**
Trends in Cell Biology, vol. 8, February 1998, pages 84-87, XP 002940006 ISSN: 0962-8924

D11: **Schwarze S et al: 'In vivo protein transduction: delivery of a biologically active protein into the mouse'**
Science, vol. 285, no. 5433, 3 September 1999, pages 1569-1572, XP 002140133 ISSN: 0036-8075

D12: **Fischer P M et al: 'Structure-activity Relationship of Truncated and**

Substituted Analogues of the Intracellular Delivery Vector Penetratin'
Journal of Peptide Research, vol. 55, no. 2, February 2000, pages 163-172, XP 000899124 ISSN: 1397-002X

D13: WO 94 04686 A

D14: US 6 306 613 B

D15: J. Silke et al.: "The anti-apoptotic activity of XIAP is retained upon mutation of both the caspase 3- and caspase 9-interacting sites"
Journal of Cell Biology, vol. 157, no. 1, 1 April 2002 (2002-04-01), pages 115-124, XP002272040 ISSN: 0021-9525

D16: Ekert P G et al.: "DIABLO promotes apoptosis by removing MIHA/XIAP from processed caspase 9"
Journal of Cell Biology, vol. 153, no. 3, 30 April 2001 (2001-04-30), pages 483-490, XP002272041 ISSN: 0021-9525

D17: Srinivasula S M et al: "A conserved XIAP-interaction motif in caspase-9 and Smac/DIABLO regulates caspase activity and apoptosis"
Nature, vol. 410, 1 March 2001 (2001-03-01), pages 112-116, XP002962286 ISSN: 0028-0836

D18: Verhagen A M et al: "HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins"
Journal of Biological Chemistry, vol. 277, no. 1, 4 January 2002 (2002-01-04), pages 445-454, XP002957689 ISSN: 0021-9258

D19: Holcik M et al: "Translation Upregulation of X-linked Inhibitor of Apoptosis (XIAP) Increases Resistance to Radiation Induced Cell Death"
Oncogene, vol. 19, no. 36, 24 August 2000 (2000-08-24), pages 4174-4177, XP008007068 ISSN: 0950-9232

D20: Verhagen Anne M et al: "Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins"
Cell, vol. 102, no. 1, 7 July 2000 (2000-07-07), pages 43-53, XP002175397 ISSN: 0092-8674

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

In the present application, the International Searching Authority has restricted the search following objections under Articles 5 and 6 PCT. The International Preliminary

Examination Authority agrees with these objections, and will limit the International Preliminary Examination accordingly.

In the present application it is to be noted, that insufficient disclosure in the sense of Article 5 PCT is given for the Smac protein / carrier entity as claimed. Indeed, the examples all refer to the expression of a plasmid. For the Smac protein / carrier entity as such, the support is limited to the mere mentioning, that "[t]he N-terminal 4 residues of Smac that are essential for inactivation of XIAP and thus for apoptosis induction, together with the 3 following residues, were linked to the protein transduction domain of the TAT protein to facilitate intracellular delivery (Smac peptide / PTD)." (Passage on page 18, lines 25-28).
Although the skilled person will known how to link stretches of amino acids together *in general*, the International Preliminary Examination Authority feels obliged to point out, that the specific choice of "stretches of amino acids" is an essential part of the invention, which should be clearly disclosed.
It is therefore highly doubtful, if the requirements of Article 5 PCT for sufficient disclosure are actually met.

Re Item IV
Lack of unity of invention

The problem underlying the present application is to provide therapeutics against cancer and autoimmune diseases. As a solution to this problem, several therapeutic possibilities are claimed:

1. Claims: 1-8, 22 The Smac/carrier entity as claimed, and a medicament for the treatment of cancer containing it.
2. Claims: 9, 10-21 Use of the Smac/carrier entity as claimed in combination with another anticancer agent, in the treatment of cancer

The technical feature these conjugates have in common resides in the fact, that the Smac protein is used or expressed.

The usefulness of Smac protein in the treatment of cancer and autoimmune diseases has, however, already been described in the prior art:
D1 and **D2** describe Smac, and the therapeutic possibilities of expression of this protein in the cytosol.
D3 mentions partial tetrapeptides of Smac.
D4 also shows cells, in which the Smac protein is expressed.
D5, finally, uses amino acids as transporters. As this documents was found when

searching for the partial sequence AVPI, it must also contain this sequence, and is therefore an anticipation for subject 1 as listed above.

Therefore, in view of these documents, this technical feature can no longer serve as special technical feature in the sense of Rule 13 PCT, linking the different subjects together. Since there is no other technical feature, that could fulfil the role of special technical feature in the sense of Rule 13 PCT, the present application lacks unity of invention, containing the subject-matters as listed.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents all disclose in a manner sufficiently clear for the skilled person to prepare fusion proteins or protein conjugates, containing a carrier part for introduction of a therapeutic protein into the cell.

D6 uses the protein VP22 for transporting through membranes. **D7** uses poly-arginine, and compares it with the TAT protein. In **D8**, penetratin is used, which is a Antennapedia. **D9** uses pAntp, partial sequence of the homeodomain of antennapedia. **D10** also uses a peptide for favouring the penetration of macromolecules into cells. **D11** uses the transduction domain of the TAT protein for transferring the enzyme β-galactosidase into all tissues of mice. It further mentions the possibility to use this process for administering proteins to patients.
In **D12**, finally, we find a discussion on the structure-activity relationship of the vector penetratin.

With regard to the combination of Smac/DIABLO with radiation therapy, the following is to be noted.
D15 describes the mechanism of action of DIABLO. According to this document, it forms part of the apoptosis-mechanism after UV irradiation, by inhibiting the XIAP. Likewise, **D16** teaches the skilled person, that DIABLO promotes apoptosis by removing XIAP from processed caspase 9. Again, the apoptosis is induced by radiation (UV).
According to **D17**, "binding of the caspase-9 linker peptide and Smac to the BIR3 domain of XIAP is mutually exclusive, suggesting that Smac potentiates caspase-9 activity by disrupting the interaction of the linker peptide of caspase-9 with BIR3. Our

studies reveal a mechanism in which binding to the BIR3 domain by two conserved peptides, one from Smac and the other one from caspase-9, has opposing effects on caspase activity and apoptosis".
D18, too mentions the implication of DIABLO in cell death (see page 445, right-hand column), as does **D20**, in which DIABLO is identified.
From these documents it is clear, that the effect of DIABLO/Smac results from the inhibition of XIAP. Since XIAP inhibits apoptosis, its inhibition is said to promote apoptosis.
These documents thus seem to anticipate part of invention number 1. As a consequence, the requirements of Article 33.2 PCT for novelty are not met.

Insofar as this objection is overcome by certain specific molecules, it is to be noted, that the effect of Smac in the treatment of cancer, and more specifically, in the sensibilisation of cancer cells to treatment, is also known from documents **D1** to **D4**. Therefore, it is very unfortunate, that the applicant has not submitted data, which would have demonstrated an unexpected effect of the specific partial sequence used. In the present case, however, the applicant has not shown, that the problem underlying the present application has actually been solved by the solutions proposed. Indeed, the example merely describes the protocol, by which this could be verified. Unfortunately, the results are not given. For this reason, it is not possible to acknowledge inventive step in the sens of Article 33.3 PCT.

Concerning the specific part of the sequence responsible for the entry into the cells it is pointed out, that these, too, are known to the skilled person. In this respect, reference is made to *inter alia* **D13**, which describes in example 2 the use of tat 37-72 (specifically mentioned in claim 8) to facilitate the entry of β-galactosidase into the cells, as shown in example 4. The same sequence is used in example 5 for HRP. The sequence used in example 7 corresponds to claim 9 + GGC, linked to ribonuclease. Other peptides are also linked to this sequence: E2 (see example 13) and VP16 (see example 16). Finally, claim 3 mentions tat 47-58 (present claim 9 plus one amino acid) under (a), and claims 16,18 and 21 mention tat 37-72 (present claim 8). In view of this document, the skilled person, wishing to modify a protein or peptide, when starting from any of **D1** to **D4** (disclosing DIABLO/Smac) to facilitate entry into cells, would certainly apply the teachings of document **D13**, thus arriving at the Smac/carrier entity presently claimed. Therefore, this technical feature cannot contribute to an inventive step in the sense of Article 33.3 PCT.

Moreover, as already mentioned under item IV, **D5** uses amino acids as transporters.

As this documents was found when searching for the partial sequence AVPI, it must also contain this sequence. Therefore this partial sequence cannot form the basis of an inventive step for invention 1.

For the specific sequence of claim 2, reference is made to **D14**, which also uses this sequence (see column 63, example 16). Again, this partial sequence is therefore insufficient support for an inventive step in the sense of Article 33.3 PCT.

With regard to the combination of the Smac/carrier entity with another anticancer agent, i.e., invention 2, the International Preliminary Examination Authority notices, that no data are available in the present application, which allows to determine, which would be the effect of this combination. The International Preliminary Examination Authority therefore has to assume, that it is a mere combination of two anti-cancer products. As it is routine practice in cancer treatment to combine more than one single agent, such a combination does not meet the requirements of Article 33.3 PCT for inventive step.